REMARKS

Claims 1-13 and 22-26 are pending in this application.

A marked-up version showing the changes made by the present amendment is attached hereto as "Version with Markings to Show Changes Made."

In the event that any fees are due in connection with this paper, please charge our Deposit Account No. 01-2340.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE SPECIFICATION:

Please amend the paragraph beginning at page 69, line 10, with the following paragraph:

Example 1

Synthesis of Ala-(Ser(SO_3H))$_5$-βAla (SEQ ID NO:3) (poly-peptide 3)

(1) Synthesis of Fmoc-Ala-(Ser)$_5$-βAla (SEQ ID NO:1)

Using 2.3 g (1.5 mmols in terms of βAla) of Fmoc-βAla-Alko Resin (100 to 200 mesh, mfd. by Watanabe Chemical Industries, Ltd.) as a starting material, Fmoc-Ala-(Ser)$_5$-βAla (SEQ ID NO:1) was synthesized by a solid phase technique according to the method (BOP/HOBt method) described in a reference (J. Org. Chem., 53, 617-624 (1988)).

Please amend the paragraph beginning at page 70, line 10, with the following paragraph:

After the introduction of all the amino acids, the resin was washed with MeOH, followed by adding thereto 20 ml of a TFA-anisole (95:5) mixed solution, and the reaction was carried out with stirring at room temperature for 1 hour to detach the desired polypeptide from the resin and remove the tBu groups (protecting groups for the hydroxyl group of Ser). After completion, of the reaction, the resin was filtered off and the filtrate was concentrated under reduced pressure. Ether was added to the concentrate to precipitate the desired compound. The precipitate was collected and then dried in a desiccator to obtain 0.84 g of Fmoc-Ala-(Ser)$_5$-βAla (SEQ ID NO:1).

Please amend the paragraph beginning at page 70, line 23, with the following paragraph:

Synthesis of Ala-(Ser(SO_3H))$_5$-βAla (SEQ ID NO:3) (polypeptide 3)

In 40 ml of DMF was dissolved 547 mg (0.67 mmol) of the Fmoc-Ala-(Ser)$_5$-βAla (SEQ ID NO:1) synthesized in (1), followed by adding thereto 30 ml of a DMF•SO_3 solution (a solution of 2.57 g of DMF•SO_3 (mfd. by Fluka Chemka-Biochemica) in 30 ml of a DMF-pyridine (4:1) mixed solution), and the reaction was carried out overnight at 4°C. To the reaction solution was added 30 ml of a DMF•SO_3 solution, and the resulting solution was subjected to reaction overnight at 4°C. After completion of the reaction, the reaction solution was added to 400 ml of acetone, and the precipitate formed was collected by filtration and dissolved in 40 ml of DMF. To the resulting solution was added 8 ml of piperidine, and the reaction was carried out with stirring at room temperature for 40 minutes to remove the Fmoc group. The reaction solution was added to 500 ml of acetone and the precipitate formed was collected by filtration, washed with acetone and ether, and then dried in a desiccator. The dried precipitate was subjected to anion-exchange chromatography and then gel filtration to obtain 210 mg of Ala-(Ser(SO_3H))$_5$-βAla (SEQ ID NO:3) (polypeptide 3).

Please amend the paragraph beginning at page 71, line 25, with the following paragraph:

Example 2

Synthesis of Ala-Tyr(SO_3H)$_3$-βAla (SEQ ID NO:13) (polypeptide 11)

Please amend the paragraph beginning at page 72, line 8, with the following paragraph:

After the introduction of all the amino acids, the resin was washed with MeOH, followed by adding thereto 50 ml of a DMF-piperidine (4:1) mixed solution, and the reaction was carried out with stirring at room temperature for 1 hour to remove the Fmoc group. The solvent was removed by filtration, after which the resin was washed with MeOH and a mixture of TFA, H_2O and m-cresol (45:5:2) was added. Then, under nitrogen gas stream, the reaction was carried out at 4°C for 16 hours to detach the desired polypeptide from the resin. The resin was removed from the reaction solution by filtration, and the filtrate was concentrated under reduced pressure, after which the desired compound was precipitated with ether. The precipitate was subjected to anion-exchange chromatography and then gel filtration to obtain 180 mg of Ala-Tyr$(SO_3H)_3$-βAla (SEQ ID NO:10) (polypeptide 11).

Please amend the following paragraph beginning at page 73, line 1, with the following paragraph:

Example 3

Synthesis of Ala-(Tyr$(SO_3H))_5$-βAla (SEQ ID NO:13) (polypeptide 14)

(1) Synthesis of Fmoc-Ala-$(Tyr)_5$-βAla (SEQ ID NO:23)

Please amend the following paragraph beginning at page 73, line 13, with the following paragraph:

After the introduction of all the amino acids, the resin was washed with MeOH, followed

by adding thereto a mixed solution of TFA, thioanisole and 1,2-ethanediol (95:5:1), and the reaction was carried out with stirring at room temperature for 1 hour to detach the polypeptide from the resin and remove the tBu groups (protecting groups for the hydroxyl group of Tyr). After completion of the reaction, the resin was filtered off and the filtrate was concentrated under reduced pressure. Ether was added to the concentrate to precipitate the desired compound. The precipitate was collected and then dried in a desiccator to obtain 1.71 g of Fmoc-Ala-$(Tyr)_5$-βAla (SEQ ID NO:23).

Please amend the paragraph beginning on page 73, line 26, with the following paragraph:

Synthesis of Ala-$(Tyr)SO_3H))_5$-βAla (SEQ ID NO:13) (polypeptide 14)

To a mixture of 0.5 g of the Fmoc-Ala-$(Tyr)_5$-βAla (SEQ ID NO:23) obtained in (1) and 3 ml of DMF was added 15 ml of a DMF•SO_3 solution (a solution of 3.2 g of DMF•SO_3 in 15 ml of DMF-pyridine (4:1) mixed solution), and the reaction was carried out overnight at 4°C, after which ether was added to the reaction solution to precipitate the reaction product. The precipitate was dissolved in 10 ml of DMF, followed by adding thereto 2.5 ml of piperidine, and the reaction was carried out with stirring at room temperature for 1 hour. To the reaction solution was added 150 ml of ether, and the precipitate formed was collected by filtration. The precipitate was dissolved in 4 ml of water and the desired compound was isolated by an ODS column liquid chromatography (column: Wakosil $_{10}C_{18}$ (2.0 ϕ x 25 cm) (mfd. by Wako Pure Chemical Industries, Ltd.); elution conditions: 10 mM AcONa (pH 6.0), 2 → 60% acetonitrile). Thus obtained fraction containing the desired compound was treated by gel filtration to obtain 203 mg of Ala-$(Tyr(SO_3H))_5$-βAla (SEQ ID NO:13) (polypeptide 14).

Please amend the paragraph beginning on page 76, line 3, with the following paragraph:

Example 6

Synthesis of Ala-(Tyr(PO_3H_2))$_5$-βAla (SEQ ID NO:22) (polypeptide 23)

Please amend the paragraph beginning on page 76, line 14, with the following paragraph:

After the introduction of all the amino acids, the resin was washed with MeOH, followed by adding thereto 50 ml of a DMF-piperidine (4:1) mixed solution, and the reaction was carried out with stirring at room temperature for 1 hour to remove the Fmoc group. After completion of the reaction, the resin was collected by filtration and washed with MeOH, and then 20 ml of a mixed solution of TFA, phenol, H_2O, thioanisole and ethanediol (33:2:2:2:1) was added. The resulting mixture was subjected to reaction with stirring at room temperature for 1 hour to detach the polypeptide from the resin. After completion of the reaction, the resin was filtered off, and ether was added to the filtrate to precipitate the desired compound. The thus obtained precipitate was subjected to anion-exchange chromatography and then gel filtration to obtain 760 mg of Ala-(Tyr(PO_3H_2))$_5$-βAla (SEQ ID NO:22) (polypeptide 23).

Please amend the paragraph beginning on page 77, line 8, with the following paragraph:

Example 7

Synthesis of 4-maleimidobutyryl-Ala-(Tyr(PO_3H_2))$_5$-βAla (SEQ ID NO:22) (polypeptide 24)

Please amend the paragraph beginning on page 77, line 19, with the following paragraph:

After the introduction of all the amino acids, the resin was washed with MeOH and treated with a TFA-anisole (95:5) mixed solution to detach the polypeptide from the resin. The resin was filtered off and ether was added to the filtrate to form a precipitate. The precipitate was subjected to an ODS column liquid chromatography (column: Wakosil $_5C_{18}$ (2.0 ϕ x 25 cm) (mfd. by Wako Pure Chemical Industries, Ltd.); elution conditions: 0.1% TFA, 0 → 10% acetonitrile) and then gel filtration to obtain 820 mg of 4-maleimidobutyryl-Ala-(Tyr(PO_3H_2))$_5$-βAla (SEQ ID NO:22) (polypeptide 24).

Please amend the table on pages 79 and 80 as follows:

TABLE 1: Structures of peptides and results of amino acid analysis and in chromatography

Peptide No.	Structure of peptide	Results of amino acid analysis				Results of ion chromatography [(amion)] (anion)	
		Ratio among amino acids				Number of sulfonic (phosphoric) group per peptide	
		Ala:	Ser:	Tyr:	βAla	Found	Calcd.
1	Ala-Ser(SO_3H)-βAla	1.0	1.0	0.0	1.0	1.0	1.0
2 (SEQ ID NO:2)	Ala-(Ser(SO_3H))$_3$-βAla	1.0	3.0	0.0	1.0	3.0	3.0
3 (SEQ ID NO:3)	Ala-(Ser(SO_3H))$_{[3]5}$-βAla	1.0	5.0	0.0	1.0	5.0	5.0
4 (SEQ ID NO:4)	Ala-(Ser(SO_3H))$_{[3]8}$-βAla	1.0	7.9	0.0	0.9	8.1	8.0
5 (SEQ ID NO:5)	(Ser(SO_3H))$_{[3]8}$-βAla	0.0	8.0	0.0	1.0	8.0	8.0
6 (SEQ ID NO: 6)	Ala-Ala-Ala-(Ser(SO_3H))$_{10}$	3.0	10.2	0.0	0.0	10.0	10.0
7 (SEQ ID NO:7)	Ala-(Ser(SO_3H))$_{20}$-βAla	1.0	18.2*	0.0	0.9	20.2	20.0
8 (SEQ ID NO:8)	Ala-(Ser(SO_3H)-Ser(SO_3H)-Ser(SO3H)-βAla)$_3$	1.0	8.8	0.0	2.9	9.0	9.0
9 (SEQ ID NO:9)	Ala-(Ser(SO_3H)-Ser(SO_3H)-βAla)$_3$	1.0	10.1	0.0	5.1	10.0	10.0
10	Ala-Tyr(SO_3H)-βAla	1.0	0.0	1.0	1.1	1.0	1.0
11 (SEQ ID NO:10)	Ala-(Tyr(SO_3H))$_3$-βAla	1.0	0.0	3.0	1.0	3.0	3.0
12 (SEQ ID NO:11)	Ala-(Tyr(SO_3H))$_4$-βAla	1.0	0.0	4.0	1.0	4.0	4.0
13 (SEQ ID NO:12)	Ala-(Tyr(SO_3H))$_4$	1.0	0.0	4.0	0.0	4.0	4.0
14 (SEQ ID NO:13)	Ala-(Tyr(SO_3H))$_5$-βAla	1.0	0.0	5.0	1.0	5.0	5.0
15 (SEQ ID NO:14)	Ala(Tyr(SO_3H))$_5$	1.0	0.0	5.0	0.0	5.0	5.0

Cont'd

Table 1 (cont'd)

16 (SEQ ID NO:15)	Ala-$(Tyr(SO_3H))_7$-βAla	1.0	0.0	5.4**	1.0	7.0	7.0
17 (SEQ ID NO:16)	Ala-$(Tyr(SO_3H))_7$	1.0	0.0	5.2**	0.0	7.0	7.0
18 (SEQ ID NO:17)	Ala-$(Tyr(SO_3H))_8$-βAla	1.0	0.0	5.3**	1.0	7.9	8.0
19 (SEQ ID NO:18)	Ala-$(Tyr(SO_3H))_8$	1.0	0.0	5.4**	0.0	8.0	8.0
20 (SEQ ID NO:19)	Ala-$(Tyr(SO_3H))_{10}$-βAla	1.0	0.0	5.2**	1.0	10.0	10.0
21 (SEQ ID NO:20)	Ala-$(Ser(So_3H))_{[2]8}$-$(Tyr(SO_3H))_5$	1.0	8.0	5.0	0.0	13.0	13.0
22 (SEQ ID NO:21)	$(Ser(SO_3H))_{[4]8}$-$(Tyr(SO_3H))_5$	0.0	8.0	4.9	0.0	13.1	13.0
23 (SEQ ID NO:22)	Ala-$(Tyr(PO_3H_2))_{[3]5}$-βAla	1.0	0.0	5.0	1.0	5.0	5.0
24 (SEQ ID NO:22)	4-Maleimidobutyryl-Ala-$(Tyr(PO_3H_2))_5$-βAla	1.0	0.0	5.0	1.0	5.0	5.0

*) The value was rather low for 20 Ser residues. From the result of ion chromatography, the structure is considered correct.

**) Less than six of the Tyr residues could be measured. It can be speculated that this result was brought about by the low water-solubility of the amino acid.

Please amend the paragraph beginning on page 85, line 21, with the following paragraph:

Example 9

Preparation of an antibody-sulfated polytyrosine combined product

(1) Preparation of 4-(p-maleimidophenyl) butytyl-Ala-$(Tyr(SO_3H))_8$-[βAa] βAla (SEQ ID NO:17)

In 500 μℓ of 0.1 M phosphate buffer (pH 7.0) was dissolved 1 mg of the Ala-$(Tyr(SO_3H))_8$-[βAa] βAla (SEQ ID NO:17) prepared in Example 4, followed by adding thereto 1.2 mg of sulfosuccinimidyl-4-(p-maleimidophenyl) butyrate (mfd. by Pierce Chemical Co.), and the reaction was carried out at 37°C for 3 hours. The reaction solution was treated with a Superdex peptide column (16 mm ID x 30 cm, mfd. by Pharmacia AB) to remove the excess reagents, whereby an aqueous solution of 0.84 mg of 4-(p-maleimidophenyl) butyryl-Ala-$(Tyr(SO_3H))_8$-[βAa] βAla (SEQ ID NO:17) was obtained (yield: 75%).

Please amend the paragraph beginning on page 86, line 18, with the following paragraph:

(3) Preparation of a combined product of Ala-$(Tyr(SO_3H))_8$-[βAa] βAla (SEQ ID NO:17) and AFP-A4-4•Fab'

In 0.1 M phosphate buffer (pH 7.0), 3.1 mg of the 4-(p-maleimidophenyl) butyryl-Ala-$(Tyr(SO_3H))_8$-[βAa] βAla (SEQ ID NO:17) obtained in (1) above and 3.1 mg of the AFP-A4-4•Fab' obtained in (2) above were reacted at 4°C for 16 hours. The reaction solution was charged into a Superdex 200 pg column (26 mm ID x 60 cm, mfd. by Pharmacia AB) to remove the excess 4-(p-maleimidophenyl) butyryl-Ala-$(Tyr(SO_3H))_8$-[βAa] βAla (SEQ ID NO:17). Then, the residue was treated with a DEAE TOYOPEARL column (10 mm ID x 2 cm, mfd. by Tosoh Ltd.) and the adsorbed fraction was recovered to obtain 1 mg of a combined product of Ala-$(Tyr(SO_3H))_8$-[βAa]

βAla (SEQ ID NO:17) and AFP-A4-4•Fab' (yield:15%).

Please amend the paragraph beginning at page 87, line 6, with the following paragraph:

Example 10

Preparation of an antibody-sulfated polytyrosine combined product

(1) Preparation of 4-(p-maleimidophenyl) butyryl-Ala-$(Tyr(SO_3H))_8$ (SEQ ID NO:18)

In 3 ml of DMF was dissolved 25 mg of the Ala-$(Tyr(SO_3H))_8$ (polypeptide 19) prepared in Example 4, followed by adding thereto 10 mg of sulfosuccinimidyl-4-(p-maleimidophenyl) butyrate (mfd. by Pierce Chemical Co.), and the reaction was carried out at room temperature for 1 hour. The reaction mixture was treated with an ODS column (column: Wakosil 5C18 (2.0 ϕ x 25 cm) (mfd. by Wako Pure Chemical Industries, Ltd.); elution conditions: 50 mM ammonium acetate pH 6, 2 - 60% acetonitrile). The thus obtained fraction containing the desired compound was concentrated to dryness to obtain 26.5 mg of 4-(p-maleimidophenyl) butyryl-Ala-$(Tyr(SO_3H))_8$ (SEQ ID NO: 18) (yield:95%).

Please amend the paragraph beginning at page 87, line 24, with the following paragraph:

NMR data of the obtained 4-(p-maleimidophenyl)butyryl-Ala-$(Tyr(SO_3H))_8$ (SEQ ID NO: 18) are shown below:

^{1}H-NMR (270 MHz, DMSO-d_6) δppm: 7.16 (s, 2H, maleimide proton)

Please amend the paragraph beginning on page 88, line 5, with the following paragraph:

It was also found that when stored at 15°C or lower, the 4-(p-maleimidophenyl)butyryl-

Ala-(Tyr(SO_3H))$_8$ (SEQ ID NO:18) obtained by the method described above can be stably stored without degradation. Thus, this compound was found to be more easily usable than the compound obtained by the method described in Example 9 (1) which was in the form of an aqueous solution and was almost completely degradable in about 24 hours.

Please amend the paragraph beginning at page 88, line 21, with the following paragraph:

(3) Preparation of a combined product of Ala-(Tyr(SO_3H))$_8$ (SEQ ID NO:18) and AFP-A4-4•Fab'

In 50 mM phosphate buffer (pH 6.5), 1 mg of the 4-(p-maleimidophenyl)butyryl-Ala-(Tyr(SO_3H))$_8$ (SEQ ID NO:18) obtained in (1) above and 11.1 mg of the AFP-A4-4•Fab' obtained in (2) above were reacted at 4°C for 16 hours. The reaction solution was fractionated by use of a POROS DEAE column (6 mm ID x 1 cm, mfd. by Perseptive Biosystems) to obtain 6 mg of a combined product of Ala-(Tyr(SO_3H))$_8$ (SEQ ID NO:18) and AFP-A4-4•Fab' (yield: 60%).

Please amend the paragraph beginning at page 89, line 12, with the following paragraph:

Although not apparent, the reason is guessed as follows: in Example 9 (1), free sulfosuccinimidyl-4-(p-maleimidophenyl) butyrate was removed using the Superdex peptide column, while in Example 10 (1), the removal was carried out using the ODS column. In detail, the following conjecture is given: since the difference in molecular weight between the polypeptide having a maleimide group introduced thereinto of the present invention and free sulfosuccinimidyl-4-(p-maleimidophenyl) butyrate was small, they could not be sufficiently separated from each other by use of the Superdex peptide column, so that free sulfosuccinimidyl-4-(p-maleimidophenyl)

butyrate reacted with Fab', resulting in a low yield of the combined product of Ala-$(Tyr(SO_3H))_8$-[βAa] βAla (SEQ ID NO:17) and AFP-A4-4•Fab'.

Please amend the paragraph beginning at page 94, line 4, with the following paragraph:

Each of Ala-$(Ser(SO_3H))_8$-βAla (SEQ ID NO:4) (polypeptide 4) and Ala-$(Tyr(SO_3H))_5$-βAla (SEQ ID NO:13) (polypeptide 14) was stored at 40°C in a buffer solution having a pH of 6 to 10, whereby their stability was investigated.

Please amend the paragraph beginning at page 96, line 21, with the following paragraph:

Anti-TSH monoclonal antibody which had been confirmed to be different in epitope from TSH-1 (hereinafter abbreviated as "TSH-2"; available from Wako Pure Chemical Industries, Ltd.) was treated into Fab' fragment (hereinafter abbreviated as "TSH-2•Fab'"). Combined products of TSH-2•Fab' and each of Ala-$(Tyr(SO_3H))_5$-βAla (SEQ ID NO:13) (polypeptide 14) and Ala-$(Ser(SO_3H))_5$-βAla (SEQ ID NO:3) (polypeptide 3) were prepared by the same procedure as described in Example 9 (3).

Please amend the paragraph beginning at page 98, line 16, with the following paragraph:

As a result of the HPLC analysis, the salt concentrations (sodium chloride concentrations) for elution of various substances was found to be as follows:

- TSH-1•Fab'-POD, and a complex of TSH-1•Fab'-POD and TSH: 0 to 0.1 M.
- a complex of TSH-1•Fab'-POD, TSH, and the combined product of TSH-2•Fab' and Ala-$(Tyr(SO_3H))_5$-βAla (SEQ ID NO:13) (polypeptide 14) : 0.5 to 1.2 M.

- a complex of TSH-1•Fab'-POD, TSH, and the combined product of TSH-2•Fab' and Ala-(Ser(SO_3H))$_5$-βAla (SEQ ID NO:3) (polypeptide 3) : 0.25 to 0.45 M.

Please amend the paragraph beginning on page 104, line 28, with the following paragraph:

There were mixed 100 μl of the antibody solution 1, 50 $\mu\ell$ of the sample and 50 $\mu\ell$ of the antibody solution 2 (containing a combined product of TSH-2•Fab' and Ala-(Ser(SO_3H))$_5$-βAla (SEQ ID NO:3) (polypeptide 3)) which had been prepared in Example 13. After standing at 25°C for 30 minutes, 20 $\mu\ell$ of the resulting mixture was subjected to measurement (analysis) by HPLC under the conditions described above. As a result, an objective antigen-antibody complex was eluted at the eluting salt concentration of an antigen-antibody complex formed when measurement of (analysis for) AFP was carried out using polypeptide 3 (data on the eluting salt concentration are also shown at a position corresponding to the abbreviation TSH on the axis of abscissa in Fig. 5). From this result, it can be seen that even in the case of a different analyte to be measured, employment of the polypeptide of the present invention as [an] a separation-improving substance makes it possible to carry out a desired measurement (analysis) by use of HPLC under definite analysis conditions.

Please amend the paragraph beginning on page 105, line 25, with the following paragraph:

Except for using anti-AFP monoclonal antibody WA-2 (hereinafter abbreviated as "AFP-WA-2"; available from Wako Pure Chemical Industries, Ltd.; different in epitope from AFP-WA-1 and AFP-A4-4) as an antibody and Ala-(Tyr(SO_3H))$_5$-βAla (SEQ ID NO:13) as a polypeptide, a combined product of AFP-WA-2•Fab' and Ala-(Tyr(SO_3H))$_5$-βAla (SEQ ID NO:13) was

prepared with the same reagents by the same procedure as described in Example 9. As liquid reagent 1, there was prepared 50 mM MES buffer (pH 6.5) containing 139 nM of the combined product, 1 mg/ml of Lens culinaris lectin (hereinafter abbreviated as "LCA"; available from Wako Pure Chemical Industries, Ltd.), 1 mM of magnesium chloride and 1 mM of calcium chloride.

Please amend the paragraph beginning at page 106, line 13, with the following paragraph:

As liquid reagent 2, there was used 50 nM MES buffer (pH 7.5) containing 147 nM of the AFP-WA-1•Fab'-POD prepared in Example 12, 156 nM of the combined product of Ala-$(Tyr(SO_3H))_8$-[βAa] βAla (SEQ ID NO:17) and AFP-A4-4•Fab' prepared in Example 9, and 0.2 (w/v)% of a poly(vinyl alcohol).

Please amend the paragraph beginning at page 108, line 11, with the following paragraph:

From the results shown in Fig. 6, the following can be seen: an antigen-antibody complex (complex 1) of AFP, the combined product of AFP-WA-2•Fab' and Ala-$(Tyr(SO_3H))_5$-βAla (SEQ ID NO:13), and AFP-WA-1•Fab'-POD was eluted at a position of 2.9 min; an antigen-antibody complex (complex 2) formed by introduction of the combined product of Ala-$(Tyr(SO_3H))_8$-[βAa] βAla (SEQ ID NO:17) and AFP-A4-4•Fab' into complex 1 was eluted at a position of 5.8 min; and these complexes are certainly separated from each other.

Please amend the paragraph beginning at page 108, line 21, with the following paragraph:

From the results shown in Fig. 6, the following can also be seen: in the case of sample 1 containing LCA-unbound AFP, complex 2 formed by the attachment of the combined product of

Ala-(Tyr(SO_3H))$_8$-[βAa] βAla (SEQ ID NO:17) and AFP-A4-4•Fab' is mainly formed as antigen-antibody complex; and in the case of sample 2 containing LCA-attachable AFP, complex 1 is mainly formed as antigen-antibody complex. These results indicate that the combined product of Ala-(Tyr(SO_3H))$_8$-[βAa] βAla (SEQ ID NO:17) and AFP-A4-4•Fab' is inhibited from reacting with AFP, by its competition with LCA.

Please amend the paragraph beginning on page 109, line 13, with the following paragraph:

The same experiment as in Example 15 was carried out except for using a combined product of AFP-WA-2•Fab' and an aspartic acid polymer with an average molecular weight of 6,000 in place of the combined product of AFP-WA-2•Fab' and Ala-(Tyr(SO_3H))$_5$-βAla (SEQ ID NO:13), and using a combined product of AFP-A4-4•Fab' and an aspartic acid polymer with an average molecular weight of 28,800 in place of the combined product of Ala-(Tyr(SO_3H))$_8$-[βAa] βAla (SEQ ID NO:17) and AFP-A4-4•Fab'. Then, the complex 1 percentage was calculated.

Please amend the paragraph beginning on page 114, line 24, with the following paragraph:

Samples were prepared by diluting with 50 mM MOPS buffer solution (pH 7.5) the AFP-A4-4•Fab' produced in Example 10, a combined product of Ala-(Tyr(SO_3H))$_8$ (SEQ ID NO:18) and AFP-A4-4•Fab', the AFP-WA2•Fab' produced in Example 15, or a combined product of Ala-(Tyr(SO_3H))$_5$-βAla (SEQ ID NO:13) and AFP-WA2•Fab', respectively, so as to make the content 1 mg/ml.

Please amend the paragraph beginning at page 115, line 5, with the following paragraph:

Each sample in an amount of 4 $\mu\ell$ was applied in the sample application wells on a side of 1% agarose-gel. The applied side was made a cathode, and electrolysis was conducted at a voltage of 200 V for 30 minutes, followed by dyeing of protein using Quick-CBB (a trade name, mfd. by Wako Pure Chemical Industries, Ltd.) to measure an Rf value of each sample.

(Results)

Rf values of the samples were as follows:

Sample	Rf value
AFP-A4-F4•Fab'	0.38
(SEQ ID NO:18) (Ala-(Tyr(SO_3H))$_8$-(AFP-A4-4•Fab')	0.66
AFP-WA2•Fab'	0.06
(SEQ ID NO:13) (Ala-(Tyr(SO_3H))$_5$-βAla)-(AFP-WA2•Fab')	0.22

Please amend the paragraph beginning on page 116, line 5, with the following paragraph:

Samples were prepared by adding 50 $\mu\ell$ of AFP solution adjusted with 50 mM MOPS buffer solution (pH 7.5) so as to make the content of AFP 0.5 mg/ml to 50 $\mu\ell$ of a solution of the combined product of Ala-(Tyr(SO_3H))$_8$ (SEQ ID NO:18) and AFP-A4-4•Fab' obtained in Example 17 (1 mg/ml) in MOPS buffer solution (pH 7.5), 50 $\mu\ell$ of a solution of the combined product of Ala-(Tyr(SO_3H))$_5$-βAla (SEQ ID NO:13) and AFP-WA2•Fab' obtained in Example 17 (1 mg/ml) in MOPS buffer solution (pH 7.5), followed by reaction at 37°C for 30 minutes.

Please amend the paragraph beginning on page 117, line 11, with the following paragraph:

Rf values of the samples were as follows:

Sample	Rf value
Antigen-antibody reaction product of	
(SEQ ID NO:18) (Ala-(Tyr)SO_3H))$_8$)-(AFP-A4-4•Fab') with AFP	0.63
Antigen-antibody reaction product of	
(SEQ ID NO:13) (Ala-(Tyr(SO_3H))$_5$-βAla)-(AFP-WA2•Fab') with AFP	0.20
AFP	0.85

As shown above, it is clear that Rf values of antigen-antibody reaction product of SEQ ID NO:18) (Ala-(Tyr(SO_3H))$_8$-(AFP-A4-4•Fab') with AFP and (SEQ ID NO:17) (Ala-(Tyr(SO_3H))$_8$-βAla)-(AFP-WA2•Fab') with AFP are, respectively, almost the same as that of (SEQ ID NO:18) (Ala-(Tyr(SO_3H))$_8$)-(AFP-A4-4•Fab') and (SEQ ID NO:13) (Ala-(Tyr(SO_3H))$_5$-βAla)-(AFP-WA2•Fab'). Thus, it is found that the negative charge of AFP [dose] does not influence Rf values of antigen-antibody reaction product of (SEQ ID NO:18) (Ala-(Tyr(SO_3H))$_8$)-(AFP-A4-4•Fab') with AFP and (SEQ ID NO:13))Ala-(Tyr(SO_3H))$_5$-βAla)-(AFP-WA2•Fab') with AFP.

IN THE CLAIMS:

1. (Amended) A polypeptide having 4 to 20 tyrosine sulfate residues [at least three acid residues derived from a strong acid].

2. (Amended) A polypeptide according to Claim 1, wherein each sulfate [said acid]

residue is bound to a reactive group in a tyrosine [an amino acid] residue constituting the polypeptide.

3. (Amended) A polypeptide represented by the formula:

$$A\text{-}(R)_m\text{-}B \qquad (I)$$

wherein m is an integer of 4 to 30 [3 or more]; 4 to 20 of [at least three] R's are tyrosine sulfate residues [the same or different, independently an amino acid residue introducing a strong acid residue thereinto via a reactive group of the amino acid residue], and the rest of R's are, the same or different, an amino acid residue having no strong acid residue, each reactive group in each side chain of the amino acid residue being able to be protected; A is a hydrogen atom, a protective group of N-terminus or an acid residue derived from a strong acid; and B is a hydroxyl group or a protective group of C-terminus.

4. (Amended) A polypeptide [according to Claim 3, which is] represented by the formula:

$$[A\text{-}(R^1)_m\text{-}B] \qquad A\text{-}(R^1)_m\text{-}B \qquad (II)$$

wherein R^1's are, the same or different, independently an amino acid residue introducing a strong acid residue thereinto via a reactive group of the amino acid residue; m is an integer of 3 to 30 [or more]; A is a hydrogen atom, a protective group of N-terminus or an acid residue derived from a strong acid; and B is a hydroxyl group or a protective group of C-terminus [and A and B are as defined in Claim 3].

5. (Amended) A polypeptide according to Claim 3, which is represented by the formula:

$[A\text{-}(R^1)_{m'}\text{-}(R^2)_n\text{-}B]$ $A\text{-}(R^3)_{m'}\text{-}(R^2)_n\text{-}B$ (III)

wherein m' is an integer of 4 to 20; [3 or more; at least three] R^3 is a tyrosine sulfate residue [R^1's are the same or different, independently an amino acid residue introducing a strong acid residue thereinto via reactive group of the amino acid residue]; each R^2 is an amino acid residue having no strong acid residue, each reactive group in each side chain of the amino acid residue being able to be protected; n is an integer of 1 to 26 [or more]; and A and B are as defined in Claim 3.

6. (Amended) A combined product of a [the] polypeptide having 3 to 30 acid residues derived from a strong acid [of Claim 1] and a substance having affinity for an analyte to be measured in a sample of body fluids or cells [derived from a living body].

7. (Amended) A compound comprising a [the] polypeptide having 3 to 30 acid residues derived from a strong acid [of Claim 1], the N-terminus of which is bound through a spacer to a maleimido group.

8. (Amended) A combined product of the compound of Claim 7 and a substance having a SH group and affinity for an analyte to be measured in a sample of body fluids or cells [derived from a living body].

9. (Amended) A compound according to claim 7, [comprising a maleimido group bound through a spacer to the n-terminus of the polypeptide of Claim 3, said compound] which is represented by the formula:

$[D\text{-}E\text{-}(R)_m\text{-}B]$ $\underline{D\text{-}E\text{-}(R^4)_m\text{-}B}$ (IV)

wherein D is a maleimido group; E is a spacer; m is an integer of 3 to 30; at least three R^4's are, the same or different, independently an amino acid residue introducing a strong acid residue therein the via a reactive group of the amino acid residue, and the rest of R^4's are, the same or different, an amino acid residue having no strong acid residue, each reactive group in each side chain of the amino acid residue being able to be protected; and B is a hydroxyl group or a protective group of C-terminus [and R, m and B are as defined in Claim 3].

10. (Amended) A compound according to claim 7 [comprising a maleimido group bound through a spacer to the N-terminus of the polypeptide of Claim 4], which is [said compound] represented by the formula:

$[D\text{-}E\text{-}(R^1)_{m'}\text{-}B]$ $\underline{D\text{-}E\text{-}(R^1)_m\text{-}B}$ (V)

wherein D is a maleimido group; E is a spacer; R^1's are, the same or different, independently an amino acid residue introducing a strong acid residue thereinto via a reactive group of the amino acid residue; m is an integer of 3 to 30; and B is a hydroxyl group or a protective group of C-terminus [and $R^{1'}$, m' and B are as defined in Claim 4].

11. (Amended) A compound according to claim 7 [comprising a maleimido group bound through a spacer to the N-terminus of the polypeptide of Claim 5], which is [said compound] represented by the formula:

$[D\text{-}E\text{-}(R^1)_{m'}\text{-}(R^2)_n\text{-}B]$ [III] $\underline{D\text{-}E\text{-}(R^1)_m\text{-}(R^2)_{n'}\text{-}B}$ (VI)

wherein D is a maleimido group; E is a spacer; m is an integer of 3 to 30; R^1's are, the same or

different, independently an amino acid residue introducing a strong acid residue thereinto via reactive group of the amino acid residue, each reactive group in each side chain of the amino acid residue being able to be protected; n' is an integer of 1 to 27; and B is a hydroxyl group or a protective group of C-terminus [and R^1, R^2, m', n and B are as defined in Claim 5].

12. (Amended) A reagent of measuring an analyte to be measured in a sample of body fluids or cells [derived from a living body], which comprises a combined product of Claim 6 [of the polypeptide of Claim 1] and a substance having affinity for the analyte.

13. (Amended) A reagent for measuring an analyte to be measured in a sample of body fluids or cells [derived from a living body], which comprises a combined product of the compound of Claim 7 and a substance having a SH group and affinity for an analyte to be measured in a sample of body fluids or cells [derived from a living body].